

Prevalence, Distribution, and Host Relationships of *Cryptosporidium Parvum* (Protozoa) Infections in the United States, 2003-2005

© By Omar M. Amin, B.S.C., M.S.C., Ph.D., USA

Abstract

Six percent of 9,856 fecal specimens from 4,928 patients from all states and the District of Columbia tested positive for *Cryptosporidium parvum* infections in 2003-2005. Annual prevalence rate was similar in all three years. Overall prevalence was lowest in winter months but peaked in May, especially in males that were relatively more frequently infected than females. Additional peaks were observed in September, 2003 and February, 2004. Frequency of infection increased with age in both sexes up to age 80-89. Twenty two percent of infected patients were asymptomatic. Intestinal symptoms were relatively more common than extra-intestinal symptoms. Singly infected males (82%) and females (77%) experienced one or more of 17 intestinal symptoms (most frequently diarrhea, bloating, and gas) and one or more of 19 extra-intestinal symptoms (most frequently fatigue, brain fog and neurological problems).

Key Words: *Cryptosporidium parvum*, USA, 2003-2005, epidemiology, annual and seasonal prevalence, age and sex distribution, multiple infections, symptomology.

Introduction

Cryptosporidium parvum is the second most common intestinal parasite found in the United States, after *Blastocystis hominis*, with prevalence rates of 4.2% and 22.9% in 2000, respectively (Amin, 2002). This is the first nation-wide epidemiological study of *Cryptosporidium parvum* dealing with its annual, seasonal, sex and age distribution, and symptomatology in the United States over a three year period between 2003 and 2005. It is the third in a number of similar large-scale studies by the same author. The first included 19 species of intestinal parasites from 5,792 fecal specimens from 2,896 patients tested in 2000 of whom 32% were infected (Amin, 2002). The second study of *Blastocystis hominis* showed 16% of 10,582 fecal specimens from 5,291 patients to be infected between 2002 and 2004. Studies of smaller populations addressing some of the areas covered by the above studies include those of Amin (1977), Kappus et al. (1994), Garcia et al. (1984) from the United States, Contreas et al. (1998) in California, and Senay and MacPherson (1990) from Ontario, Canada.

Materials And Methods

A total of 9,856 fecal specimens from 4,928 patients (two specimens per patient) were collected and transported to Parasitology Center, Inc. (PCI) (Tempe, AZ) in Proto-Fix™ in plastic vials provided in mailable kits by Alpha-Tec Systems, Inc. (Vancouver, WA). Specimens were collected throughout the United States between January 2003 and December 2005 following physicians orders. Tests were ordered either as part of routine medical examinations or when patients experienced changes in bowel habits, energy level, or normalcy after a foreign trip, bad meals, or other exposures. Specimens were processed and stained in CONSED™ prior to examination according to manufacture's (Alpha-Tec Systems, Inc.) directions and Amin (2000). This procedure was previously used in 10,358 specimens by 1998, and was described, validated at PCI and evaluated and compared with other methods (Amin, 2000). The number of specimens found positive (number of individuals and of species of parasites) was significantly higher than in other methods compared, e.g., formalin-ethyl acetate or trichrome stain. These observations were supported by findings of other observers (Allen and Frankel, 1997; Jensen et al, 2000). The Proto-Fix™ CONSED™ system involves filtering of fixed specimens, mixing with CONSED™ and ethyl acetate, vortexing, centrifugation, decanting all but the fecal plug, and mixing with CONSED™ diluting reagent. The plug is then transferred to and mounted on a slide for examination (Amin, 2000). Processed specimens were stored for up to 3 days before examination. All microscopic evaluations and identification were made of processed and stained specimens by the same observer (OMA) blinded to patient information, e.g., symptoms travel, etc. to insure consistency. Positive results were quantified (number of organisms per high-power field on a scale of 1 to 4) from duplicate samples from each patient. Symptoms were marked at time of stool collection but may have been experienced for up to four recorded weeks.

Results

The annual prevalence of infections with *Cryptosporidium parvum* varied between 5% and 6% between 2003 and 2005. The overall prevalence in all three years was 6% in 4,928 examined patients. Total prevalence of 6% in 1,552 examined males was slightly higher than that

in 3,376 females (5%) (Table 1). The overall seasonal prevalence was lowest in the colder months of the year and highest in the spring especially in males during April and May (Table 1). However, additional peaks were also observed in September, 2003 and February, 2004.

The youngest age group (age 0-9) had the lowest prevalence rates (0-3%). The rates increased with increased age to 7-9% up to age 80-89 years (Table 2).

A total of 59 multiply infected patients (18% of 100 males and 23% of 179 females) was concurrently infected with six other species of protozoans. *Blastocystis hominis* was identified in 46 (78%) of current infections (Table 3). Twenty two percent of singly infected males and females were asymptomatic.

The 64 symptomatic singly infected males and 108 females (Table 3) experienced one or more intestinal and extra-intestinal symptoms. The most frequently reported intestinal symptoms were diarrhea (25% in males, 19% in females), cramps (16%, 17%), bloating (11%, 19%), and gas (14%, 15%) Extra-intestinal symptoms (Table 5) were less common than intestinal symptoms and more frequently included fatigue (27%, 18%), brain fog (14%, 15%), and neurological symptoms (12%, 11%). Men experienced more diarrhea and fatigue than women who reported more bloating and skin rash than men.

Discussion

The overall prevalence of 6% observed in this study is considerably greater than those reported in surveys from North America (0.6-4.3%) and Europe (1-2%) but lower than those reported from Asia, Australia, Africa, Central America, and South America (3-20%) (Current and Garcia, 1991; Garcia, 2001). *Cryptosporidium parvum* appears to be underdiagnosed in the western hemisphere; its seroprevalence is 25% to 35% in Europe and North America and up to 64% in South America (Ungar et al., 1990). In clinics along the Texas-Mexico border, 70% of sampled children and households were infected with *C. parvum* (Leach et al., 2000 and Redlinger et al., 2002, respectively). Twenty four percent of immunocompromised patients with diarrhea in developing areas were reported to suffer from cryptosporidiosis compared with 14% in developed countries (Martins and Guerrant (1995). *Cryptosporidium* oocysts were observed in 27% of drinking water samples taken from 66 surface water treatment plants in 14 states and one Canadian province (LeChevallier et al., 1991). Other studies indicate that *Cryptosporidium* oocysts are present in 65-97% of surface water in the U.S. (Blanchfield, 1996). The effect of urban vs. rural, developing vs. developed countries, transmission patterns in different environmental settings, and epidemic years should be taken into account. For instance, in tropical Africa, highest prevalence reached 72.8% (genotype 1) and 18.4% (genotype 2) in Ugandan children during the rainy months of April to June (Tumwine et al., 2003). In the 1993 Milwaukee epidemic, 403,000 people were

exposed and infected out of a population of 1.6 million (25.2%) (Mackenzie et al., 1994) and in the Carroll County, Georgia outbreak of 1987, *C. parvum* was identified in stools of 39% of persons examined with estimated attack rates of 54% and 40% within the city of Carrollton and the county, respectively (Hayes et al., 1989).

The annual prevalence of *C. parvum* in US populations tested at PCI showed a slight increase from 4.2% in 2000 (Amin, 2002) to 5% in 2003 and 6% in 2004 and 2005 (Table 1). More dramatic oscillations in annual prevalence were observed in two studies of immune compromised HIV-infected US populations. In New Orleans, *C. parvum* prevalence increased from 2.9% in 1989 to 20% in 1994 then decreased to 5.3% in 1998 (n = 6,913 patients) (Inungu, 2000). In southern California, the prevalence of microsporidiosis cases (species not identified) was 8.8% in 1993 9.7% in 1994, 6.6 % in 1995, and 2.9 % in 1996 (n = 8,439 stool specimens) (Conteas et al., 1998). These results suggest the constant presence of microsporidia in the environment. Factors related to such oscillations in annual prevalence may be related to rain fall. They however, remain to be clarified. A recent CDC surveillance of the general US population noted slight changes in *C. parvum* prevalence from 1/100,000 in 1999 (n = 2,769), to 1.1/ 100,000 in 2000 (n = 3,128), to 1.3/100,000 in 2001 (n = 3,3787), to 1.0/100,000 in 2002 (n = 3,016). The number of outbreak cases was 353,429,389, and 207, respectively (Hlavsa, et al., 2005). Those minor changes in prevalence are more or less similar to the rather stable rates observed in our present PCI study.

The low overall seasonal prevalence during the colder months (October to March) and the greater rates during the warmer months especially during April and May in males (Table 1) in 2003-2005 were similar to those observed in 2000 (Amin, 2002). Peaks in *C. parvum* prevalence appear to correspond with warmer seasons in temperate and tropical climates especially when associated with rain. During the rainy seasons, the runoff from cattle farms readily contaminate surface waters feeding into treatment plants as happened during the March-April, 1993 Milwaukee outbreak. The author actually knows the official responsible for the contamination of both Milwaukee plants involved in that outbreak. This type of seasonal waterborne fecal contamination was also reported by various observers throughout the world, e.g., Hlavasa et al. (2005) in the United States, Inungu et al. (2000) in New Orleans, Bern et al. (2002) in Peru, Howe et al. (2002) in England, Chai et al. (2001) in Korea, Tumwine et al. (2003) in Uganda, Mahgoub et al. (2004) in Jordan, Bern et al. (2000) in Guatemala, Katsumata et al. (1998) in Indonesia, and Nchito et al. (1998) in Zambia.

Non-water sources (food stuffs, drinks, animal to person contact, person to person contact, and exposure to contaminated recreational water, etc.) were estimated to represent about 50% of total exposures (Anonymous, 1999).

Higher percentages of such exposures are known especially in arid desert countries with little rainfall. In such environments, the highest seasonal prevalences are observed in the cold season. Such seasonality was well documented in the desert country of Kuwait with highest prevalence rates between November and April when 77% of infections occurred. *Cryptosporidium parvum* is highly endemic in Kuwait where 58% of 1-19 year old children are infected (Sulaiman et al, 2005). Similar seasonal periodicity was observed in Kenya where the highest prevalences were noted between November and February and human to human transmission was the main mode of infection, (Gatei et al, 2006)

Slightly higher prevalence rates in males than in females were observed in this study (Table 1) and in a few other studies, e.g. Mahgoub (2004) And Gatei et al. (2006). Risk factors such as zoonotic recreational and professional exposure may be involved.

The overall prevalence rates were lowest in children but progressively increased in male and female patients in older age groups up to 80-89 years old between 2003- and 2005 (Table 2). This pattern is consistent with similar observations by Chai et al. (2001), Inungu et al. (2000), Steinberg et al, (2004), Naumova et al. (2003), and Leach et al. (2000). The elderly appear to be at a greater risk of severe disease due to *Cryptosporidium* infection, with shorter incubation period than has been previously reported in all adults, and with a high risk of secondary person-to-person transmission (Naumova et al., 2003). That risk was 19% in 5.8% of Brazilian households with identified index cases (Newman et al., 1994). One year after primary challenge with *C. parvum* oocysts, 19 healthy immunocompetent adults were rechallenged. Fewer subjects shed oocysts after the second exposure than after the first exposure even though the rates of diarrhea were comparable (Okhuyesen et al., 1998).

Children, however appear to have higher prevalence rates than adults in developing countries (Black, 1996) e.g., in Indonesia (Katsumata et al., 1998), India (Nagamani et al., 2001), Guatemala (Bern et al., 2000; Laubach et al., 2004), and Nigeria (Okafor and Okunji, 1996). Under these conditions, repeated exposure to infection in the first three years of life, even without diarrhea (Bern et al., 2000) and the considerably higher sero-positive results than those obtained from prospective assessment of stool samples (Robin et al., 2001) can account for that pattern. In addition, the finding that *C. parvum* infections were considerably more common in breast-fed Zambian children compared to other children (Nchito et al., 1998) may have implications regarding transmission between nursing mothers and new born children affecting the higher prevalence rates in this age group. Prevalence rates were similar in breastfed (26%) and non-breastfed (29%) 10 month old children (n=1779) from Uganda (Tumwine et al., 2003).

Concurrent infections of 18% and 23% of male and female patients with six other species of parasites were observed, with *Blastocystis hominis* making up the majority (78%) these infections (Table 3). These figures are somewhat similar to those observed in 2000 when 121 *C. parvum* cases of 2,896 tested patients (4.2%) included 31 concurrently infected cases (26%) (Amin, 2002). *Blastocystis hominis* made up most of these infections also. Mixed infections appear to be more prevalent in developing countries, e.g., 70-72 % in Venezuela (Chacin-Bonilla and Sanchez-Chavez, 2000) indicating common exposure to human fecal contamination. The most common coinfection in Kenya was with *Entamoeba histolytica*/*E. dispar*; no *B. hominis* was reported (Gatei et al., 2006).

Twenty two percent of singly infected males and females were asymptomatic (Table 3) compared to 30% of singly infected patients in 2000 (Amin, 2002). The prevalence rates of asymptomatic patients varied widely between human populations in various geographical regions. In endemic areas of developing nations, repeated exposure, especially in young children, appear to provide sequential events of immune competency affecting lack of symptoms. For instance, prevalence of asymptomatic cases was 60.6% in Venezuela (Chacin-Bonilla and Sanchez-Chaves, 2000), 63% in Peruvian children (Checkley et al., 1997), 86% along the Mexican / US border (Redlinger et al., 2002), and 50% in Tanzania (Haupt et al., 2005) and in Zambia (Kelly et al., 2004). Garcia (2001) indicated, on the other hand, that in industrial cultures like the USA, "all patients appear to have symptoms" which is an obvious overstatement. In our experience, there are three types of *C. parvum* clinical pictures. The first is the asymptomatic carrier who is highly immunocompetent and resilient to infection. Newman et al. (1994) demonstrated evidence of antibodies (IgM or IgG) to *C. parvum* in 94.6% of his subjects. A second level of immunocompetency produces an acute short-term, self-limited diarrhea lasting approximately two weeks. Lastly, immunocompromised patients will suffer a wide range of chronic symptoms that may become progressively worse in time and may cause death when complicated by sequelae. It has been shown that the difference between these clinical pictures is related to the infective oocyst dose and virulence, and the degree of host systemic or enteric immunocompetency which determines the extent of structural and functional damage to the intestinal track (Clark and sears, 1996; Current, 1988; Current and Garcia, 1991; Garcia, 2001; Martins and Guerrant, 1995). The possible contribution of enterotoxins (Guarino et al., 1995) can not be overlooked. Rivasi et al. (1999) demonstrated the correlation between the severity of *C. parvum* infection and the degree of histologic alterations.

The most frequently reported intestinal symptoms in singly infected patients was diarrhea (Table 4). It is practically the only symptoms used by observers to characterize clinical status in symptomatic patients. Secretory diarrhea

in *C. parvum* patients develops when intestinal absorption is impaired or secretion is enhanced and the lining enterocytes are damaged or killed (Clark and Sears, 1996). Bloating and abdominal cramps were the next commonly reported intestinal symptoms (Table 4). In a review of 586 cases from 36 surveys, Fayer and Ungar (1986) reported considerably higher prevalence of diarrhea (92%), nausea and vomiting (51%), abdominal pain (45%), and low grade fever (36%). Similar symptoms were reported by other observers but were not quantified, e.g., Current and Garcia (1991). On the other hand, no association was found between *C. parvum* infections and diarrhea in communities along the United States-Mexico border where most cases (86%) were asymptomatic (Redlinger et al., 2002). Similar prevalences of symptoms were noted in anthroponotic cryptosporidiosis in Kenya (Gatei et al., 2006). Chronic fatigue was the most commonly reported extra-intestinal symptom (21%) followed by brain fog (15%) and neurological symptoms (12%), among others (Table 5). Other extra-intestinal symptoms not observed in our study include hepatitis, pancreatitis, other respiratory problems, and damage to biliary epithelia (Garcia, 2001) as well as stunting of growth especially in symptomatic children from developing countries (Checking et al., 1997; Tumwine et al., 2003).

Acknowledgements

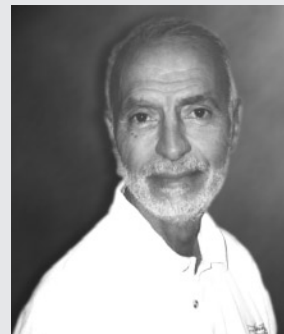
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About the Author



Dr. Amin earned his B.Sc. in Botany and Zoology and M. Sc. in Medical Entomology from Cairo University, and Ph.D. in Parasitology from Arizona State University. His professional training started at the US Naval Medical Research Unit #3 (NAMRU-3), Cairo as a Research Assistant in Medical Zoology. His post-doctoral work was at Old Dominion University,

Norfolk. He subsequently worked at the University of Wisconsin as a Professor of Parasitology, Allied Health and Biology for 20 years. In 1992, he founded the Institute of Parasitic Diseases (IPD) (for research and clinical testing of human parasites). He has a joint laboratory facility in Mexico and Mali, West Africa as well as continued research association with NAMRU-3 in Cairo.

Dr. Amin is a nationally and internationally recognized authority in Parasitology. He specializes in the systematics, ecology and pathology of protozoans, helminths and arthropods. He has published over 145 major articles/book chapters/teaching videos on parasites from North America, Peru, Chile, North, South and East Africa, Persian Gulf, the Middle East, Taiwan, Japan, Thailand, Vietnam, Inner Mongolia (China), Russia and India. He is an active lecturer on parasitological and related disease topics to health care professional, allied health workers and medical students in seminar and workshop settings.

Dr. Amin is an active member in the American Society of Parasitologists (and its Rocky Mountain affiliate), British Society of Parasitology, Entomological Society of America, Helminthological Society of Washington, American Microscopical Society, Microbiology and Arizona Homeopathic and Integrative Medical Association.

Table 1: Seasonal prevalence of *Cryptosporidium parvum* in the United States patients in 2003-2005

Month	Number of patients infected / number examined (prevalence)					
	2003	2004	2005	Total	Males	Females
January	2 / 121 (2%)	4 / 111 (4%)	6 / 119 (5%)	12 / 351 (3%)	3 / 119 (3%)	9 / 232 (4%)
February	3 / 127 (2%)	17 / 148 (11%)	5 / 131 (4%)	25 / 406 (6%)	9 / 140 (6%)	16 / 266 (6%)
March	3 / 157 (2%)	13 / 151 (9%)	8 / 145 (6%)	24 / 453 (5%)	7 / 141 (5%)	17 / 312 (5%)
April	7 / 182 (4%)	8 / 133 (6%)	12 / 122 (10%)	27 / 437 (6%)	14 / 131 (11%)	13 / 306 (4%)
May	14 / 138 (10%)	13 / 137 (9%)	11 / 160 (7%)	38 / 435 (9%)	18 / 146 (12%)	20 / 289 (7%)
June	1 / 117 (1%)	11 / 166 (7%)	14 / 159 (9%)	26 / 442 (6%)	10 / 147 (7%)	16 / 295 (5%)
July	8 / 129 (6%)	3 / 119 (3%)	9 / 127 (7%)	20 / 375 (5%)	9 / 113 (8%)	11 / 262 (4%)
August	9 / 131 (7%)	11 / 121 (9%)	5 / 149 (3%)	25 / 401 (6%)	10 / 134 (7%)	15 / 267 (6%)
September	10 / 106 (10%)	9 / 172 (5%)	11 / 134 (8%)	30 / 412 (7%)	7 / 130 (5%)	23 / 282 (8%)
October	5 / 140 (4%)	2 / 135 (1%)	8 / 173 (5%)	15 / 448 (3%)	5 / 137 (4%)	10 / 311 (3%)
November	4 / 112 (4%)	7 / 153 (5%)	8 / 148 (5%)	19 / 413 (5%)	2 / 114 (2%)	17 / 299 (6%)
December	8 / 132 (6%)	5 / 127 (4%)	5 / 96 (5%)	18 / 355 (5%)	6 / 100 (6%)	12 / 255 (5%)
Total	74 / 1592 (5%)	103 / 1673 (6%)	102 / 1663 (6%)	279 / 4928 (6%)	100 / 1552 (6%)	179 / 3376 (5%)

Table 2: Sex and age distribution of *Cryptosporidium parvum* in United States patients in 2003-2005

Age	Number of patients infected / number examined (prevalence)					
	2003	2004	2005	Total	Males	Females
0-9	2 / 94 (2%)	3 / 135 (2%)	3 / 126 (2%)	8 / 355 (2%)	8 / 241 (3%)	0 / 114 (0%)
10-19	1 / 71 (1%)	5 / 87 (6%)	4 / 58 (7%)	10 / 216 (5%)	5 / 126 (4%)	5 / 90 (6%)
20-29	5 / 145 (3%)	4 / 111 (4%)	8 / 147 (6%)	17 / 403 (4%)	9 / 142 (6%)	8 / 261 (3%)
30-39	12 / 247 (5%)	12 / 231 (5%)	10 / 225 (4%)	34 / 703 (5%)	13 / 189 (7%)	21 / 514 (4%)
40-49	16 / 395 (4%)	28 / 396 (7%)	31 / 431 (7%)	75 / 1222 (6%)	25 / 328 (8%)	49 / 894 (5%)
50-59	28 / 341 (8%)	24 / 412 (6%)	24 / 371 (6%)	76 / 1124 (7%)	24 / 275 (9%)	52 / 849 (6%)
60-69	6 / 172 (3%)	15 / 171 (9%)	13 / 170 (8%)	34 / 513 (7%)	9 / 147 (6%)	25 / 366 (7%)
70-79	3 / 70 (4%)	10 / 77 (13%)	7 / 74 (9%)	20 / 221 (9%)	4 / 58 (7%)	15 / 163 (9%)
80-89	1 / 24 (4%)	0 / 15 (0%)	1 / 24 (4%)	2 / 63 (3%)	1 / 14 (7%)	1 / 49 (2%)
90-99	0 / 2 (0)	0 / 0 (0%)	0 / 1 (0%)	0 / 3 (0%)	0 / 0 (0%)	0 / 3 (0%)
Age unknown	0 / 31 (0%)	2 / 38 (5%)	1 / 36 (3%)	3 / 105 (3%)	1 / 32 (3%)	2 / 73 (3%)
Total	74 / 1592 (5%)	103 / 1673 (6%)	102 / 1663 (6%)	279 / 4928 (6%)	100 / 1552 (6%)	179 / 3376 (5%)

Table 3: Distribution of single and multiple infections with *Cryptosporidium parvum* among symptomatic and asymptomatic male and female patients, 2003-2005

	Males	Females	Total
Infected	100	179	279
Singly infected (%)	82 (82%)	138 (77%)	220 (79%)
Multiply infected (%)*	18 (18%)	41 (23%)	59 (21%)
Symptomatic singly infected	64/82 (78%)	108/138 (78%)	172/220 (78%)
Asymptomatic singly infected	18/82 (22%)	30/138(22%)	48/220 (22%)

*** Total multiple infections included mostly *Blastocystis hominis* in 46 of 59 infected patients (78%). Four patients were also concurrently infected with *Entamoeba coli*, three with *Cyclospora cayetanensis*, two with *Entamoeba hartmanni*, two with *Ascaris lumbricoides*, and one with *Dientamoeba fragilis*.**

Table 4: Frequency of reported intestinal symptoms in single infections with *Cryptosporidium parvum* in symptomatic patients between 2003 and 2005

Symptoms	Occurrences (% of total occurrences)		
	Males	Females	Total
Abdomal pain /Cramps	16 (16%)	19 (19%)	35 (18%)
Acid reflux	4 (4%)	4 (2%)	8 (2%)
Bloating	11 (11%)	35 (19%)	46 (16%)
Blood in stool	0	1 (1%)	1 (<1%)
Burning	6 (6%)	5 (3%)	11 (4%)
Constipation	6 (6%)	19 (10%)	25 (9%)
Diarrhea	25 (25%)	35 (19%)	60 (21%)
Food allergies	4 (4%)	4 (2%)	8 (2%)
Gas	14 (14%)	29 (15%)	43 (15%)
IBS / Colitis	3 (3%)	3 (2%)	6 (2%)
Indigestion	7 (7%)	8 (4%)	15 (5%)
loss of appetite	1 (1%)	0	1 (<1%)
Malabsorption	1 (1%)	0	1 (<1%)
Mucus in stool	0	2 (1%)	2 (1%)
Vomiting	0	3 (2%)	3 (1%)
Yeast overgrowth	0	2 (1%)	2 (1%)
Total	98 (100%)	184 (100%)	283 (100%)

* The actual number of patients involved can not be shown because of the multiplicity of symptoms often reported by individual symptomatic patients.

Table 5: Frequency of reported extra- intestinal symptoms in single infections with *Cryptosporidium parvum* in symptomatic patients between 2003 and 2005

Symptoms	Occurrences (% of total occurrences)		
	Male	Females	Total
Allergies	2 (3%)	3 (2%)	5 (2%)
Asthma/cough	0	3 (2%)	3 (1%)
Bladder/vaginal/tongue infections	0	8 (5%)	8 (4%)
Brain fog	11 (14%)	22 (15%)	33 (15%)
Chills	3 (4%)	0	3 (1%)
Circulatory problems	0	3 (2%)	3 (1%)
Crohn's disease	0	1 (<1%)	1 (<1%)
Dehydration/sweating	1 (1%)	1 (<1%)	2 (1%)
Fatigue	21 (27%)	27 (18%)	48 (21%)
Hair loss	0	2 (1%)	2 (1%)
Headache	3 (4%)	6 (4%)	9 (4%)
Insomnia	5 (7%)	2 (1%)	7 (3%)
Muscle pain	3 (4%)	10 (7%)	13 (6%)
Neurological problems*	9 (12%)	17 (11%)	26 (12%)
Nausea/ malaise	5 (7%)	13 (9%)	18 (8%)
Rash	4 (5%)	17 (11%)	21 (9%)
Rhinitis/sinusitis	1 (1%)	4 (3%)	5 (2%)
Swelling	2 (3%)	3 (2%)	5 (2%)
Weight changes	6 (8%)	6 (4%)	12 (5%)
Total	76 (100%)	148 (100%)	224 (100%)

* Neurological problems included blurred vision, depression, lack of concentration, loss of motor skills, memory loss, itchy skin and tingling sensations.

